Phytotaxa 461 (4): 233–242
https://www.mapress.com/j/pt/

Article

ISSN 1179-3155 (print edition)
PHYTOTAXA
ISSN 1179-3163 (online edition)

https://doi.org/10.11646/phytotaxa.461.4.1

Trigonotis motuoensis (Boraginaceae), a new species from Xizang, China

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Abstract

Trigonotis motuoensis, a distinct new species endemic to Xizang, China, is described and illustrated. It can be easily distinguished from all other known congeneric species by the ebracteate inflorescences, broadly spatulate calyx lobes and obliquely trigonous-tetrahedral nutlets that have flat bottom surface and triangular abaxial surfaces. Our molecular phylogenetic analysis based on *nr*ITS confirmed that it belongs to *Trigonotis* sect. *Elongatae*. An updated key to the species of *Trigonotis* from Xizang is also provided.

Keywords: bract, morphology, taxonomy, tetrahedral nutlets, *Trigonotis floribunda*

Introduction

Trigonotis Steven (1851: 603), with ca 60 species, is mainly distributed in East & Southeast Asia, and West to Southeast Russia (Kadereit & Bittich 2016, Mabberley 2017). There are about 43 species in China (37 species endemic), with the main center of diversity in Southwest China, and 9 species distributed in northern China (Zhu *et al.* 1995, Wang 2007, 2010). *Trigonotis* species mainly inhabit shrub-forest margins, rocky crevices, moist areas and roadsides (Zhu *et al.* 1995). The genus is mainly characterized by tetrahedral nutlets and imbricated corolla lobes. Three sections have been recognized based on the morphology of the nutlets: *T.* sect. *Hemisphaerocarpae* C.J.Wang (1982: 33), *T.* sect. *Elongatae* Ohwi (1937: 115) and *T.* sect. *Trigonotis* (Wang 1982).

During recent field surveys in 2019, an interesting population of *Trigonotis* was found in the forest of Beibeng Village, Motuo (Mêdog) County in Southeast Xizang (Tibet). This population could be easily distinguished from any other known congeneric species by its nutlets with flat bottom, broadly spatulate calyx lobes and white throat appendages. After reviewing literature and comparing specimens (A, BM, BNU, CDBI, E, IBSC, K, KUN, KYO, NAS, P, PE, WUK), we found that the obliquely trigonous-tetrahedral nutlets of this population was similar to species in *T.* sect. *Trigonotis*, but could be distinguished by ebracteate scorpioid cyme. In addition, the morphology of leaves and inflorescence of this population was more similar to *Trigonotis floribunda* I.M.Johnston (1952: 70) in *T.* sect. *Elongatae*, but could be easily distinguished by the morphology of nutlets. Therefore, this population was thought to represent a new species, and hereafter named *Trigonotis motuoensis*.

Methods and methods

Morphological observations

Images of morphological features were taken by Nikon digital camera in the field. Twenty individuals of the new species were sampled for morphological study. All types (or type photos) of accepted names and their synonyms

Accepted by Hai He: 27 Sept. 2020; published: 9 Oct. 2020

in *Trigonotis* from Xizang and adjacent regions were examined. Nutlets were photographed by stereoscope (ZEISS V8).

Taxon sampling

In order to determine the systematic position of the new species, 12 species of *Trigonotis* from China and Japan were compiled. *Eritrichium splendens* Kearney ex W.Wight (1902: 410) was selected as an outgroup. Voucher information and GenBank accession numbers are provided in Table 1.

TABLE 1. Taxa used in molecular analyses.

Taxon	Location	Voucher	Accession no.
Trigonotis motuoensis Q.R.Liu & X.M.Xu	Xizang, China	X. M. Xu & D. H. Liu BNU2019XZ182 (BNU)	MN821514
Trigonotis amblyosepala Nakai & Kitag.	Beijing, China	J. F. Huang 2010004 (XJBI)	JX976814
Trigonotis bracteata C.J.Wang	Xizang, China	Yalung Zangbo Exp. 1126 (BNU)	MN821522
Trigonotis brevipes Maxim.	Kumamoto, Japan	K. Ikeda *et al.* (2013, unpubl.)	AB808587
Trigonotis cinereifolia C.J.Wang	Xizang, China	J. C. Hao XZ14478 (BNU)	MN821520
Trigonotis floribunda I.M.Johnst.	Sichaun, China	X. M. Xu & Q. Hu BNU2019SC210 (BNU)	MN821516
Trigonotis guilielmi (A.Gray) Gürke	Japan	Azuma 2001 (BSB)	KU927795
Trigonotis heliotropifolia Hand.-Mazz.	Yunnan, China	X. M. Xu & Q. Hu BNU2019YN015 (BNU)	MN821523
Trigonotis microcarpa (DC.) Benth. ex C.B.Clarke	Xizang, China	L. Wei & J. C. Hao 15563 (BNU)	MN821521
Trigonotis peduncularis (Trev.) Benth. ex Baker & Moore	Xizang, China	L. Wei & J. C. Hao 15216 (BNU)	MN821524
Trigonotis radicans Steven	Jilin, China	H. Hertel 22497 (M)	KF849110
Trigonotis rockii I.M.Johnst.	Xizang, China	L. Wei & Y. He BNUXZ2016413 (BNU)	MN821517
Trigonotis tibetica (C.B.Clarke) I.M.Johnst.	Xizang, China	L. Wei & J. C. Hao 15439 (BNU)	MN821518
Eritrichium splendens Kearney ex W.Wight	Alaska, USA	Solstad & Elven 03/1216 (O)	JQ388501

DNA extraction, amplification and sequencing

Genomic DNA of the new species was extracted from silica-gel dried samples of leaf tissue using the Plant Genomic DNA Kit (Tiangen, Beijing, China) following the manufacturer's instructions. The internal transcribed spacer (ITS) region was amplified with the universal primer pair ITS1 and ITS4 (White *et al.* 1990). The DNA amplification reactions followed Winkworth *et al.* (2002). Products of amplification reactions were sequenced with an ABI3730XL automated DNA sequencer (BGI Tech. Solutions Beijing Liuhe Co., Limited, Beijing, China).

Sequence alignment and phylogenetic analyses

Sequences were automatically aligned with MAFFT (Katoh 2002). Three methods, Maximum parsimony (MP), Maximum Likelihood (ML) and Bayesian inference (BI), were used to analyze phylogenetic relationships. ML and BI trees were performed with Randomized Axelerated Maximum Likelihood (RAxML)-HPC2 (Stamatakis 2014) and MrBayes 3.2.2 on XSEDE (Ronquist *et al*. 2012) respectively, from the Cyber-infrastructure for Phylogenetic Research (CIPRES) Science Gateway (Miller *et al*. 2010). The parameter settings for ML and BI followed Wei *et al.* (2017). In ML phylogenetic trees, bootstrap (BS) was calculated with 1000 replicates (Stamatakis *et al.* 2008) and the value was considered to be significant for nodes with BS ≥ 70%. In BI phylogenetic trees, nodes with posterior probability support (PP) ≥ 0.95 were considered to be significant. Additionally, the MP analysis were performed with PAUP 4.0 (Swofford 2002). Most parsimonious trees were estimated using heuristic searches with 1,000 random addition-sequence replicates and tree bisection and reconnection (TBR) branch swapping. The bootstrap support values were calculated using parsimony and 1000 heuristic-search replicates. And, the clades were considered to be significant with BS ≥ 70% in MP phylogenetic trees. The phylogenetic trees were illustrated using TreeGraph 2 (Stöver & Müller 2010).

TABLE 2. Comparison of *T. motuoensis*, *T. microcarpa* and *T. floribunda.*

Characters		*T. motuoensis*	*T. microcarpa*	*T. floribunda*
Inflorescence	Bracts	ebracteate	bracts at base only	ebracteate
Leaves	Shape	blade elliptic or ovate	ovate to oblong	elliptic or ovate-lanceolate
	Size (cm)	2–8 × 0.5–2	1.5–5 × 0.5–2	2–5 × 1–2
Calyx	Lobe shape	broadly spathulate	lanceolate	narrowly oblong
	Size (mm)	1–1.2 × 0.5–0.7	2–2.5 × 1–1.6	ca. 1.5 × 0.6
Corolla	Lobe shape	broadly obovate	broadly ovate	suborbicular
	Throat appendages	white	yellow	yellow
Nutlets	Shape	obliquely trigonous-tetrahedral	obliquely trigonous-tetrahedral	inverted trigonous-tetrahedral
	Surface	densely tuberculate	densely tuberculate	glabrous
	Carpophore	sessile	short	sessile
	Abaxial surfaces	triangular, slightly concave	broadly triangular	triangular, concave
	Adaxial surfaces	2 lateral surfaces subequal, bottom surface flat	2 lateral surfaces subequal, bottom surface slightly convex	3 surfaces subequal

Results

After comparing specimens, we found that the new species could be easily distinguished from allied species distributed in Xizang and adjacent regions by the ebracteate scorpioid, such as *Trigonotis bracteata* C.J.Wang (1980: 254), *T. cinereifolia* C.J.Wang (1980: 254), *T. clarkei* R.R.Mill (1996: 122), *T. gracilipes* I.M.Johnston (1937: 9), *T. microcarpa* (DC in Candolle 1846: 123) Benth. ex C.B.Clarke (1883: 172), *T. multicauli*s Benth. ex C.B.Clarke (1883: 172), *T. ovalifolia* Benth. ex C.B.Clarke (1883: 172), *Trigonotis peduncularis* (Trevirano 1816: 147) Benth. ex Baker & Moore (1879: 384), *Trigonotis rockii* I.M.Johnston (1925: 47), *Trigonotis rotundifolia* Benth. ex C.B.Clarke (1883: 172) and *Trigonotis tibetica* (C.B.Clarke 1883: 165) Johnston (1925: 48). In addition, *Trigonotis hookeri* Benth. ex C.B.Clarke (1883: 173) and the new species both were ebracteate, but the former could be easily distinguished from the new species by its acute apex of leaves, oblong calyx lobes, pedicels long to 5 mm and nutlets long to 2 mm.

The new species combined obliquely trigonous-tetrahedral nutlets and ebracteate inflorescences. Even though the nutlets morphology of the new species was similar to species in *T.* sect. *Trigonotis*, they could be easily distinguished by inflorescences and the shapes of leaves. The characters of inflorescences and leaves of the new species were similar to *T. floribunda*, but the difference of nutlets morphology between these two species was obvious. A detailed comparison of *T. microcarpa* in *T.* sect. *Trigonotis*, *T. floribunda* and the new species was provided in Table 2. Nutlets of these three species were showed in Figure 1.

In the systematic analysis, two major clades were formed within *Trigonotis* (Fig. 2). In Clade A (ML_{BS} = 100, MP_{BS} = 100, PP = 1), the new species, *T. motuoensis*, was sister group to *T. floribunda*, which were collected from Southeastern Sichuan. Clade B (ML_{BS} = 90, MP_{BS} = 98, PP = 0.98) contained most species from China and two species from Japan.

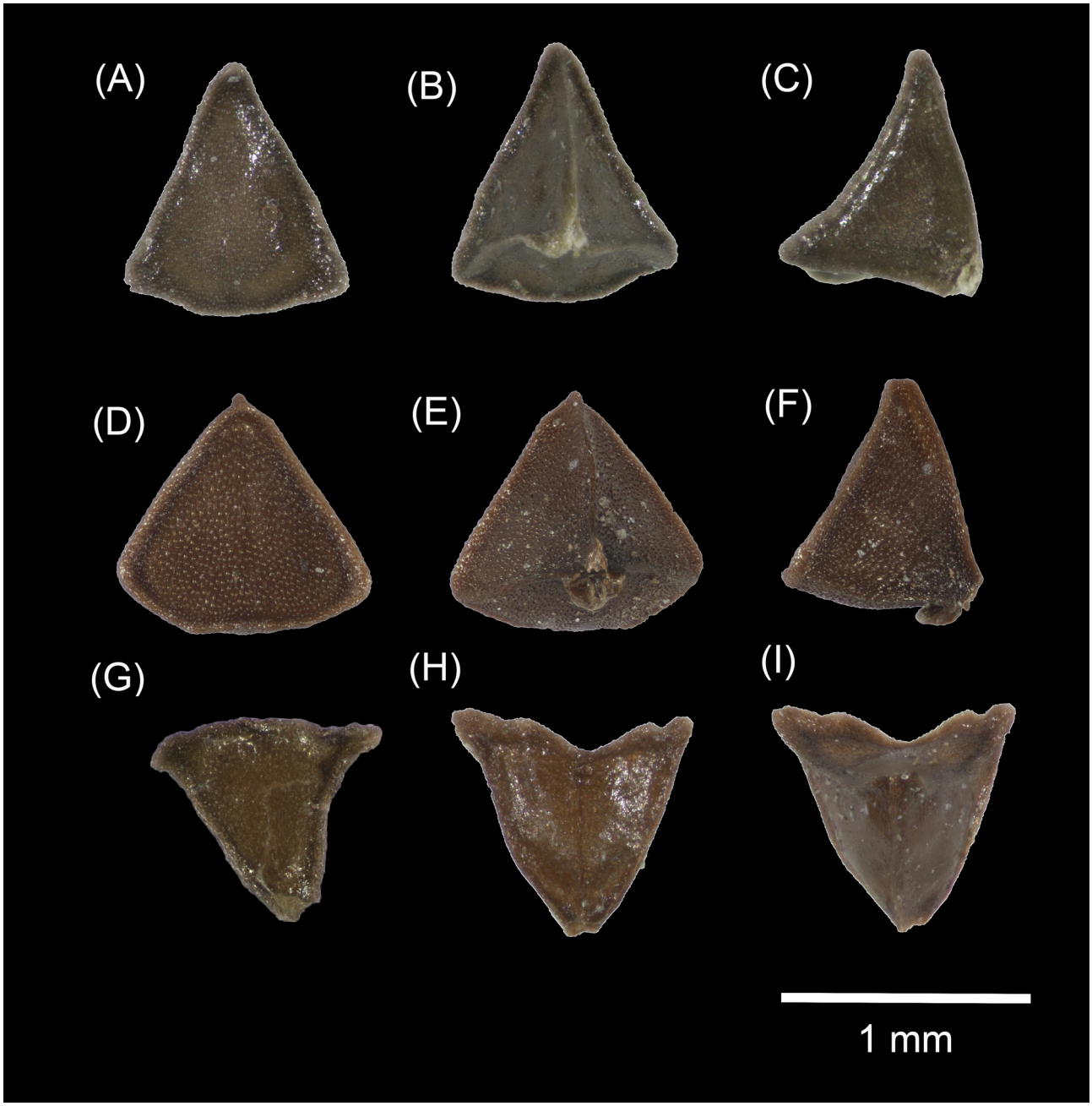

FIGURE 1. Comparison of nutlets morphology of three species. (A)–(C) *Trigonotis motuoensis sp. nov.* (A) abaxial view, (B) adaxial view, (C) lateral view. (D)–(F) *T. microcarpa*, (D) abaxial view, (E) adaxial view, (F) lateral view. (G)–(I) *T. floribunda*, (G) abaxial view, (H) adaxial view, (I) lateral view.

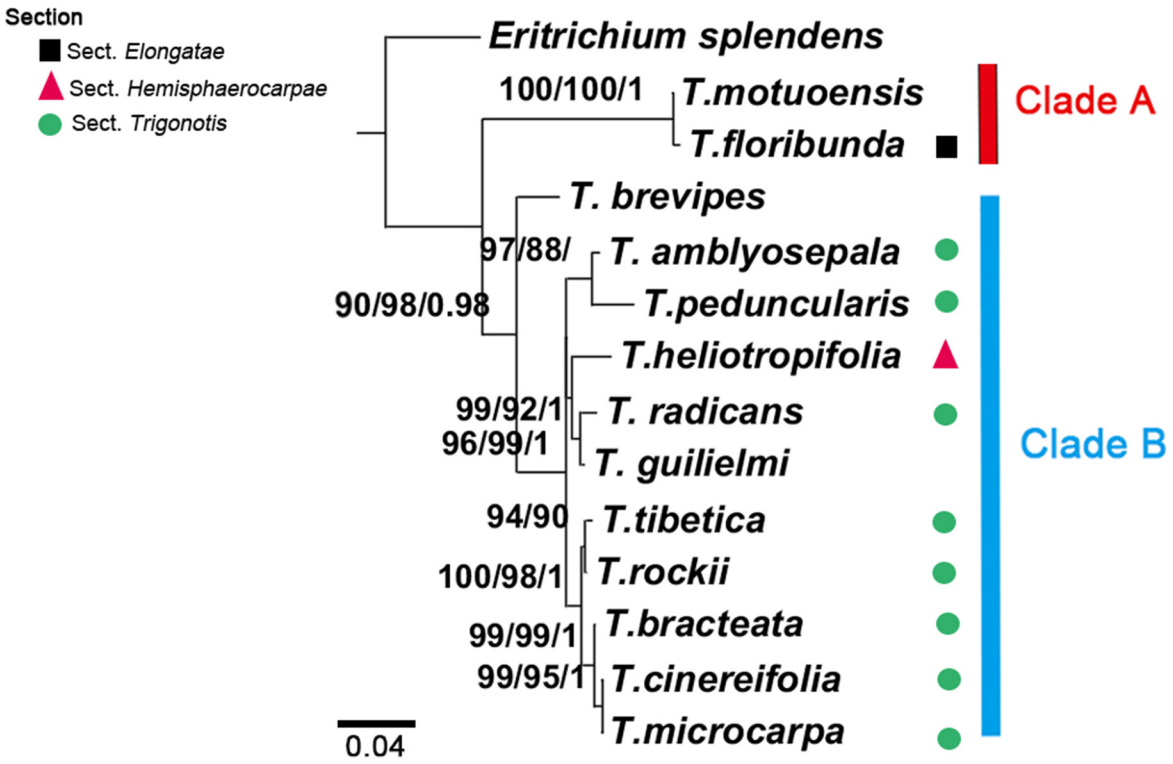

FIGURE 2. RAxML tree topology with ML_{BS} (left), MP_{BS} (middle) and PP (right) values shown on the branches, based on *nr*ITS sequences; The scale represents the substitution rate of bases.

Taxonomy

Trigonotis motuoensis Q.R.Liu & X.M.Xu, s*p. nov*. (Figs. 3–4**).**

Type:—CHINA. Xizang: Motuo County, Beibeng Village, 29°20′47.64″N, 95°9′53.25″E, in the forest, elev. ca 1500 m, 28 May 2019, *X. M. Xu & D. H. Liu BNU2019XZ182* (holotype BNU0049249!; isotypes BNU!).

Diagnosis:—*Trigonotis motuoensis* is characterized by the combination of ebracteate inflorescences, broadly spatulate calyx lobes and obliquely trigonous-tetrahedral nutlets that have flat bottom surface and triangular abaxial surfaces. It is similar to *T. floribunda* in inflorescences and leaves, but it differs by its obliquely trigonous-tetrahedral nutlets (vs. inverted trigonous-tetrahedral) and broadly spatulate calyx lobes (vs. narrowly oblong calyx lobes). Additionally, in nutlets morphology, *Trigonotis motuoensis* is similar to *T. macrocarpa* (Fig. 1) and other species in *T.* sect. *Trigonotis*, but its inflorescences and leaves are distinctly different from them (Table 2).

Herbs perennial. Rhizomes with many fibrous roots. Stems usually single, rarely many, erect or upper parts ascending, 15–30 cm tall, strigose, usually branched. Basal leaves: petiole 1.5–3 (–4.5) cm long; blade elliptic or ovate, 2–5 cm long, 1.3–2 cm wide, strigose, rounded to broadly cuneate at base, rounded to obtuse at apex, margin entire, mid-vein conspicuous, lateral veins inconspicuous. Stem leaves: petiole of lower leaves 0.6–2 cm long, upper ones shortly petiole to subsessile; blade elliptic or ovate, 2.5–8 cm long, 0.5–2 cm wide, gradually decreasing in size from lower to upper, strigose, cuneate at base, acuminate at apex, its margin entire and mid-vein conspicuous, lateral veins inconspicuous. Scorpioid cyme terminal or axillary on upper stems, peduncles 0.5–2 cm long or sessile, usually dichotomously branched, ebracteate, with 20–40 flowers, 5–10 cm long, circinate when young, gradually elongated, strigose. Pedicels slender, ca. 2 mm long, strigose. Calyx lobes broadly spathulate, acute at apex, 1–1.2 mm long, 0.5–0.7 mm wide, densely strigose. Corolla light blue, ca. 2 mm long, ca. 2.5 mm wide, with 5 semi-lunar, white appendages on throat, lobes broadly obovate, ca 1 mm long, rounded at apex. Anthers ovate, ca. 0.3 mm long. Nutlets 4, obliquely trigonous-tetrahedral, dark brown, ca 1 mm long, acutely 3-ribbed, abaxial surfaces triangular and slightly concave, adaxial 2 lateral surfaces subequal, bottom surface smaller and flat, densely tuberculate, glabrous, shiny, sessile.

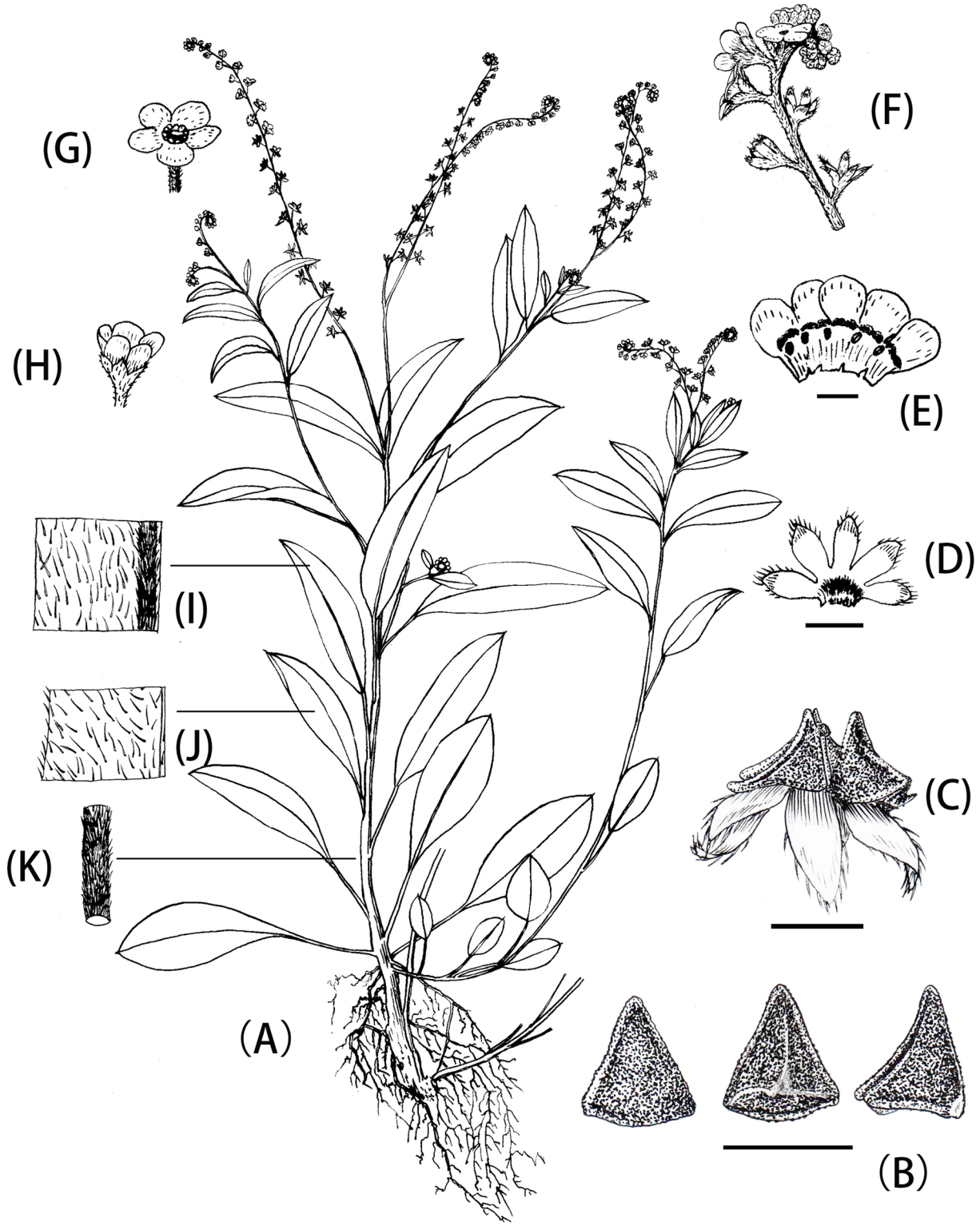

FIGURE 3. *Trigonotis motuoensis sp. nov.* (A) habit, (B) nutlets (abaxial surfaces, adaxial surfaces, lateral surfaces), (C) four mature nutlets, (D) opened calyx, (E) open corolla showing anther and throat appendage, (F) inflorescence, (G) flower (frontal view), (H) flower (lateral view), (I) abaxial surface of leaves, showing setae, (J) adaxial surface of leaves, showing setae, (K) stem, showing hairs. Scale bar = 1 mm.

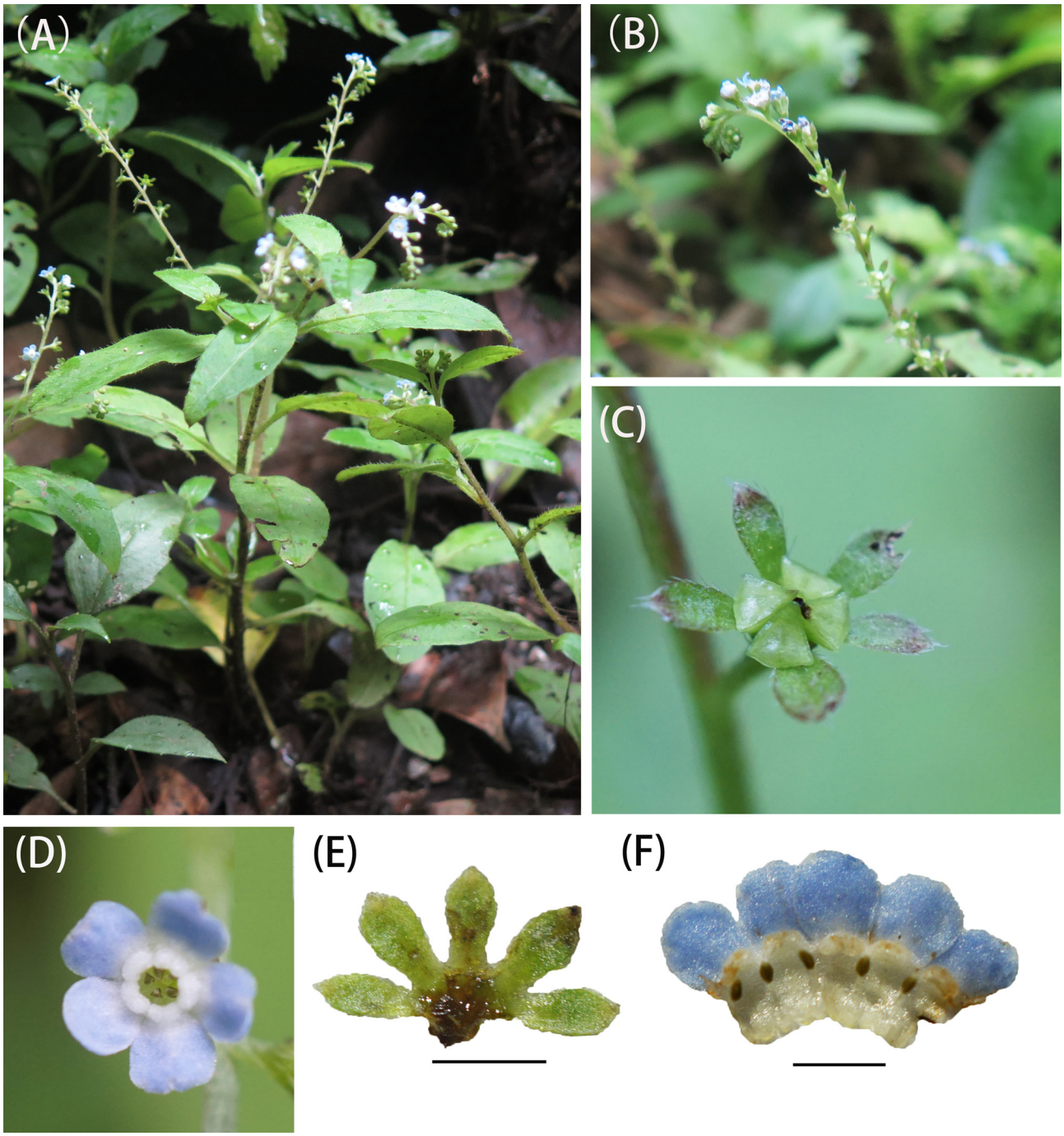

FIGURE 4. *Trigonotis motuoensis sp. nov.* (A) habit, (B) inflorescence, (C) flower in frontal view, (D) opened calyx, (E) opened corolla with stamens and appendages. Scale bar = 1 mm.

Distribution and habitat:—*Trigonotis motuoensis* is endemic to the Southeast of Xizang, China. As best we know, it has only been collected at the type locality, Beibeng Village, Motuo County. It usually grows in the forest, at elevation ca. 1500 m.

Phenology:—Flowering and fruiting from May to July.

Conservation status:—Although *T. motuoensis* is so far known only from its type locality. The only population we discovered includes more than 500 mature individuals. According to the IUCN red list categories and criteria (IUCN 2019), *T. motuoensis* should be categorized as a 'Least Concern (LC)' species. Further investigation and research into the range of this species would be needed to more fully assess the conservation status. More floristic surveys are needed in similar habitats to find other populations of this species.

Etymology:—The specific epithet '*motuoensis*' refers to the name of the county, Muotuo, where the holotype was collected.

Additional specimens examined

***Trigonotis bracteata* C.J.Wang:**—CHINA. Xizang, Bomi, 2100 m, Jul. 1965, *D. Y. Hong & J. S. Ying 650802* (PE!); Cuona, 3700 m, Jul. 2014, *J. C. Hao XZ14465* (BNU!); Motuo, Oct. 2012, *Yalung Zangbo Exiped. 1126* (BNU!).

***Trigonotis cinereifolia* C.J.Wang:**—CHINA. Xizang, Bomi, 2030 m, Jul. 1965, *K. Y. Lang & Y. T. Zhang 773* (PE!); Cuona, 4000 m, Jul. 2014, *J. C. Hao XZ14307* (BNU!).

***Trigonotis clarkei* R.R.Mill:**—INDIA. Singalelah, Darjeeling, 3352 m, Oct. 1870, *C. B. Clarke 12718* (K!); NEPAL. Gopan Than, *N. Wallich 927* (K!).

***Trigonotis gracilipes* I.M.Johnst.:**—CHINA. Sichuan, Jiulong, 3200 m, Jun. 1979, *Q. Q. Wang 20252* (CDBI!); Kangding, Aug. 1963, *K. J. Guan et al. 1415* (PE!); Luding, Jun. 1980, *Q. Q. Wang & Z. A. Liu 22392* (CDBI!); Xizang, Chayu, Aug. 1935, *Q. W. Wang 66056* (PE!); Linzhi, 3600 m, Jul. 2012, *C. Wang LZ058* (BNU!); Milin, 4100 m, Jun. 2011, *C. Wang s.n.* (BNU!).

***Trigonotis hookeri* Benth. ex C.B.Clarke:**—INDIA. Mount. Khasia, Mofleng, 1828 m, Aug. 1850, *J. D. Hooker & T. Thomson 1991* (K!).

***Trigonotis microcarpa* (DC.) Benth. ex Clarke:**—CHINA. Xizang, Cuona, 2800 m, Aug. 2015, *J. C. Hao & L. Wei 15563* (BNU!); Motuo, 1500 m, May 2019, *D. H. Liu & X. M. Xu BNU2019XZ181* (BNU!); Yunnan: Binchuan, 2747 m, Aug. 2013, *J. C. Hao & K. Wu YN002* (BNU!); Gongshan, Aug. 1940, *K. M. Feng 7015* (PE!); Jinping, Oct. 2010, *Q. R. Liu & S. Y. Meng* (BNU!); Pingbian, May 1954, *P. Y. Mao 4282* (WUK!).

***Trigonotis multicaulis* Benth. ex C.B.Clarke:**—INDIA. Sikkim Lateng, 3505 m, Jul. 1849, *J. D. Hooker s.n*. (K!).

***Trigonotis ovalifolia* Benth. ex C.B.Clarke:**—NEPAL. Gopan Than, *N. Wallich 927* (K!).

***Trigonotis peduncularis* (Trev.) Benth. ex Baker & Moore:**—CHINA. Sichuan, Daocheng, 3716 m, Jul. 2015, *J. C. Hao 15083* (BNU!); Dege, 2965 m, Jul. 2015, *J. C. Hao 15129* (BNU!); Xizang, Chayu 3472 m, Jun. 2019, *D. H. Liu & X. M. Xu BNU2019XZ280* (BNU!); Linzhi, 3200 m, Jul. 2014, *J. C. Hao XZ14241* (BNU!); Naqu, 4300 m, Jul. 2015, *J. C. Hao* 15200 (BNU!).

***Trigonotis rockii* I.M.Johnst.:**—CHINA. Xizang, Chayu, 4100 m, Jun. 1973, *Z. C. Ni 441* (PE!); Cuona, 4255 m, Aug. 2015, *J. C. Hao & L. Wei 15569* (BNU!); Dingri, 4000 m, Jul. 2016, *L. Wei & Y. He BNUXZ2016381* (BNU!); Yunnan: Deqin, 4684 m, Aug. 2015, *J. C. Hao 15585* (BNU!).

***Trigonotis rotundifolia* Benth. ex C.B.Clarke:**—INDIA. *N. Wallich 930* (BM!).

***Trigonotis smithii* Banerjee** (1966: 325)**:**—INDIA. Sikkim Llonok, 4419 m, Jul. 1909, *W. W. Smith & G. H. Cave 1811* (K!).

***Trigonotis tibetica* (Clarke) I.M.Johnst.:**—CHINA. Sichuan, Aba, 3625 m, Aug. 2007, *D. E. Boufford et al 39527* (PE!); Luding, 1800 m, May 1981, *G. H. Xu 25326* (CDBI!); Muli, 2500 m, Jul. 1978, *G. Hu 7722* (CDBI!); Xizang, Basu, 4000 m, Aug. 2015, *J. C. Hao 15577* (BNU!); Bomi, Jun. 1980, *Z. C. Ni et al 0118* (PE!); Jilong, 3700 m, Jul. 2015, *J. C. Hao & L. Wei 15439* (BNU!); Linzhi, 3040 m, Jul. 1965, *K. Y. Lang & Y. T. Zhang 1108* (PE!); Milin, 2770 m, Jun. 2019, *D. H. Liu & X. M. Xu BNU2019XZ367* (BNU!).

Discussion

The most striking characters of the new species described here are the ebracteate inflorescences and obliquely trigonous-tetrahedral nutlets. Besides, it has broadly spatulate calyx lobes and white throat appendages differing from the allied species.

Traditionally, as for infrageneric classification of *Trigonotis*, the characters of the nutlets and inflorescences are prevailing (Ohwi 1937, Popov 1953, Wang 1982). The most important characters of *T.* sect. *Trigonotis* are the obliquely trigonous-tetrahedral nutlets and inflorescences with bracts (Wang 1982). According to nutlets morphology, the new species should be placed in *T.* sect. *Trigonoti*s, however the inflorescences of the new species are ebracteate distinguished from the section. In contrast, the inflorescences of *T.* sect. *Elongatae* are ebracteate, but the morphology of nutlets of this section is inverted trigonous-tetrahedral (Wang 1982). Therefore, when we only use morphology characters, it is very difficult to decide which section the new species should belong to. In addition, our molecular systematic result clearly indicated that the new species was phylogenetically closer to *T. floribunda* from Sichuan, rather than species in *T.* sect. *Trigonotis* from Xizang. Based on these results, we suggest that *T. motuoensis* should be placed in *T.* sect. *Elongatae*. This indicate that the character of inflorescences is possibly more important than nutlets morphology for the division of sections in *Trigonotis*. Ohwi (1937) indicated that whether inflorescences with bracts and the position of bracts were significant for the classfication of *Trigonotis*, but the author only focused on 11 species of *Trigonotis* in Japan. Therefore, the diagnostic characters used for the classification of *Trigonotis* need further research.

Key to the species of *Trigonotis* from Xizang, China

1. Inflorescences without bract, bottom surface of nutlets flat ..... *T. motuoensis*
- Inflorescences with bract, bottom surface of nutlets slightly convex 2
2. Corolla 7–8 mm in diameter ..... *T. rockii*
- Corolla 2–5 mm in diameter 3
3. Junction of calyx tube with pedicel distinctly thickened, clavate in fruit 4
- Junction of calyx tube and pedicel not clavate 5
4. Leaves narrowly elliptic, stem leaves sessile; corolla ca. 3 mm in diameter ..... *T. tibetica*
- Leaves various,mainly spatulate; corolla ca. 2 mm in diameter ..... *T. peduncularis*
5. Inflorescences bracteate; stems single, erect to spreading, without runners ..... *T. bracteata*
- Inflorescences bracteate only at base 6
6. Stems and leaves gray, densely strigose; nutlets sessile ..... *T. cinereifolia*
- Stems and leaves green, sparsely strigose; nutlets with peduncle 7
7. Nutlets peduncle curved; lateral vein of abaxial leaf surface inconspicuous ..... *T. gracilipes*
- Nutlets peduncle short and not curved; lateral vein of abaxial leaf surface conspicuous ..... *T. microcarpa*

Acknowledgements

We would like to express gratitude to the curators of the herbaria A, BM, CDBI, E, IBSC, K, KUN, KYO, NAS, P, PE, WUK for assistance and access to specimens. This research was supported by the National Natural Science Foundation of China (no. 31770213).

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